How I Do It—Optimal Methodology for Multidirectional Analysis of Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration Samples

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Background: Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive modality with a high diagnostic yield for mediastinal lymph node staging of patients with lung cancer. Although limited to the use of needle aspiration during EBUS-TBNA, aspirates has been shown to be useful for molecular analysis. However, the ideal methodology of the specimen handling during EBUS-TBNA has not been described.

Methods: Based on our institutional experience and review of the literature, we describe the optimal methodology for multidirectional analysis of EBUS-TBNA samples.

Results: EBUS-TBNA specimens can be used for molecular analysis such as epidermal growth factor receptor (EGFR), Anaplastic lymphoma kinase (ALK) and V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS) mutations when processed properly. Rapid on-site cytology is invaluable during the assessment of the aspirated during EBUS-TBNA.

Discussion: Obtaining adequate samples through a non-surgical biopsy technique for pathologic diagnosis as well as molecular analysis will be of immediate importance for personalized management of lung cancer. EBUS-TBNA is an ideal approach that allows combined pathological and molecular analysis of metastatic lymph nodes.

Key Words: Endobronchial ultrasound-guided transbronchial needle aspiration, Microsampling, Molecular testing.

METHODS

Endobronchial Ultrasound-Guided Transbronchial Needle Aspiration

The convex probe EBUS (BF-UC180F, Olympus, Tokyo, Japan) used is integrated with a convex probe transducer (7.5 MHz) on the tip of the bronchoscope that scans parallel to the insertion direction of the bronchoscope. The ultrasound images are processed using a dedicated ultrasound scanner (Olympus, EU-ME1). A dedicated 22-gauge or 21-gauge needle can be used to perform TBNA (NA-201SX-4022, NA-201SX-4021, Olympus).

Lymph Node Sampling

After penetration into the lymph node, the internal stylet is used to clean out the internal lumen, which may become clogged with bronchial membrane. The internal stylet is then removed. Negative pressure can be applied with a syringe on demand. The needle is usually moved back and forth inside the lesion of interest 10 to 15 times over 30 to 60 seconds. The needle is then withdrawn into the catheter and removed from the bronchoscope, and the internal stylet is used once again to push out the aspirated material. Without rapid on-site cytology, it has been reported that three aspirations per lymph node station or two aspirations with one tissue core is recommended.

Specimen Handling and Sample Preparation for Molecular Testing

The aspirated material is pushed out on to the slide glass. The core is then placed on a filter paper to absorb limited number of centers. Nonsurgical modalities for obtaining tumor specimens are particularly important for the management of patients with lung cancer, because the majority of the patients will have advanced disease at the time of first presentation and, therefore, not eligible for surgery. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) contributes to the resolution of this issue, because samples obtained by EBUS-TBNA can be used for multiple molecular testing. This report reviews and suggests optimal methodology of EBUS-TBNA specimen handling to get the most out of an aspirate, for both molecular testing and pathologic diagnosis.
excess blood. Part of the core can be freshly stored using stabilizing solutions for extraction of RNA. We have used the Allprotect Tissue Reagent (Qiagen, Dusseldorf, Germany) following the manufacturer’s instructions with excellent results, although other products may serve the same function. The tissue processed with the filter paper is fixed with 10% neutral buffered formalin, and a formalin-fixed paraffin-embedded (FFPE) sample is made. The remaining material is smeared onto glass slides for both air-dried smears and immediately fixed with 95% ethanol smears. The air-dried smears are stained by Diff-Quik staining for rapid on-site cytology or Giemsa stain solution. The ethanol-fixed smears are sent for Papanicolaou staining. In addition, the needle is rinsed into a tube containing normal saline for flow cytometry and/or microbacterial examination. This is further centrifuged for preparing cell pellet for DNA and/or RNA extraction. The FFPE samples are usually stained with hematoxylin and eosin for histologic diagnosis. In some cases, immunohistochemistry can be performed if needed. The FFPE sample can be used for several kinds of in situ hybridization. In addition, DNA and/or RNA can also be extracted from FFPE samples that are applicable for genetic analysis (Fig. 1). The successful histologic diagnostic rate has been reported to range from 65.1 to 81.0%.3,4 Using this technique, the success rate of obtaining histologic cores when lymph node metastasis is present is assumed to be more than 80%.

**DISCUSSION**

EBUS-TBNA has been shown to have a high diagnostic yield for mediastinal lymph node staging in patients with lung cancer.5 In addition, samples obtained by this minimally invasive techniques have been used for molecular analysis.6,7 To improve the yield of EBUS-TBNA, it is important to optimize the methodology of specimen handling during the procedure. For cytologic evaluation, smears can be processed by air-dry method and ethanol fixation.8 Histologic cores can be used to make FFPE samples for histologic diagnosis, which would enable pathologists to perform immunohistochemistry.3 Flow cytometry and in situ hybridization are extremely helpful for the assessment of lymphoma (Fig. 2). 8 Molecular testing has been highlighted for the management of patients with lung cancer, especially in directing targeted therapy with EGFR tyrosine kinase inhibitors.9,10 EGFR gene mutation assessment and other molecular markers, such as ALK fusion gene alteration, are of importance for current management decisions.11 Furthermore, there is a possibility of genetic difference between the primary tumor and metastatic sites because of the tumor heterogeneity.12,13 Molecular assessment of metastatic sites (lymph nodes) by EBUS-TBNA may lead to optimal treatment of patients with advanced lung cancer (Fig. 2).

The development of a safe and precise modality that enables the acquisition of sufficient amount of high-quality...
tissue without surgery has become increasingly important in the molecularly targeted therapy era. EBUS-TBNA is an ideal approach that allows molecular analysis of metastatic lymph nodes in patients with lung cancer. Combined molecular and pathologic analyses of the specimen obtained by EBUS-TBNA will aid in the optimal management of patients with lung cancer and preclude the need for more invasive diagnostic tests.

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REFERENCES


